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Article

High site fidelity does not equate to population genetic structure for common goldeneye and Barrow's goldeneye in North America

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Delineation of population structure provides valuable information for conservation and management of species, as levels of demographic and genetic connectivity not only affect population dynamics but also have important implications for adaptability and resiliency of populations and species. Here, we measure population genetic structure and connectivity across the ranges of two sister species of sea ducks: Barrow's goldeneye *Bucephala islandica* and common goldeneye *B. clangula*. We use two different marker types: 7–8 nuclear microsatellite loci assayed across 229 samples and 3678 double digest restriction-site associated DNA sequencing (ddRAD-seq) loci assayed across 61 samples. First, both datasets found no evidence of genetic structure within common or Barrow's goldeneye, including between North American and European samples of common goldeneye. These results are in contrast with previous mitochondrial DNA, band recovery and telemetry data which suggest that goldeneyes are structured across their range. We posit that the discordance between autosomal genetic markers and other data types suggests that males, possibly subadult males, may be maintaining genetic connectivity across each species' respective ranges. Next, although mate choice consequences resulting from inter-specific brood parasitism was hypothesized to cause some level of gene flow between goldeneye species, we only identified a single F1 hybrid with no further evidence of contemporary or historical gene flow. Despite ddRAD-seq demographic analyses which recovered an optimum evolutionary model of split-with-migration (i.e. secondary contact), estimates of gene flow were <<1 migrant per generation in both directions. Together, we conclude that either strong ecological barriers or assortative mating are likely playing a role in preventing further backcrossing. Finally, demographic analyses estimated a relatively deep divergence time between Barrow's goldeneye and common goldeneye of ~1.6 million years before present and suggests that the genomes of both species have been under similar evolutionary constraints.

Keywords: *Bucephala*, dispersal, hybridization, population genetic structure, population genomics, sea ducks, waterfowl

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Introduction

Delineating population boundaries is critical for understanding demography, migratory connectivity and population dynamics, which are all important factors in effective management and conservation (Lande 1988, Supple and Shapiro 2018, Sonsthagen et al. 2019). However, directly documenting regional population structure and individual dispersal events can be challenging, especially for species that reside in remote landscapes and occupy large distributions across their annual cycle (Lavretsky et al. 2016, Sonsthagen et al. 2019). Quantifying genetic structure and rates and directionality of gene flow within a species can provide valuable information on these various stochastic and demographic factors. Additionally, contrasting patterns of genetic structure among marker types (e.g. mtDNA versus autosomal) help to identify evolutionary mechanisms (e.g. sex-bias in philopatry) or landscape factors (Manel et al. 2003, Lowe and Allendorf 2010) that are necessary to understand when creating efficient conservation strategies (Epps et al. 2007, Richardson et al. 2016).

Hybridization is much more prevalent than previously thought, occurring in ~16% of all avian taxa (Ottenburghs et al. 2015, Ottenburghs 2019), and most commonly in waterfowl (i.e. ~50% of Anatidae hybridize; Johnsgard 1960, Ottenburghs et al. 2015). Additionally, as species modify their distributions in response to shifting climate patterns (Van der Putten et al. 2010) and more localized anthropogenic changes to the landscape (Gauthier et al. 2015, Ellis-Felege et al. 2017), shifts in microhabitat selection may provide additional opportunities for inter-specific hybridization. Hybridization and more specifically gene flow also play an important role in how genetic diversity is distributed across the landscape (Hitchings and Beebee 1997, Morrissey and De Kerckhove 2009), and can affect local population dynamics and viability (Eadie et al. 1998, Eadie and Anstey 1999). Moreover, it is essential to quantify rates of hybridization, as persistent gene flow is now a major conservation concern and can lead to a loss of diversity through lineage fusion (Kearns et al. 2018) or genetic swamping (Rhymer and Simberloff 1996, Wells et al. 2019).

Barrow's goldeneye *Bucephala islandica* and common goldeneye *B. clangula* are both relatively long-lived sea ducks (Tribe Mergini), with an age of sexual maturation at 2–3 years and an average lifespan of 7–9 years (Milonoff et al. 2002). Additionally, both species are secondary cavity nesters that primarily nest in wetlands surrounded by boreal and coniferous forests, and winter along coastal bays and estuaries (Fig. 1A; Johnsgard 1978, del Hoyo et al. 1992). While common goldeneye have a Holarctic distribution, Barrow's goldeneye are more restricted; ~90% of their total population occurs in northwest North America (i.e. the Pacific Coast and mountain regions of Alaska south to northern California; Johnsgard 1978, del Hoyo et al. 1992). Similar to other sea ducks, females of both species exhibit some fidelity to breeding sites (Savard and Eadie 1989, Eadie et al. 1995, Pöysä et al. 1997, Ludwichowski et al. 2002). In British Columbia, web-tagged Barrow's goldeneye and common

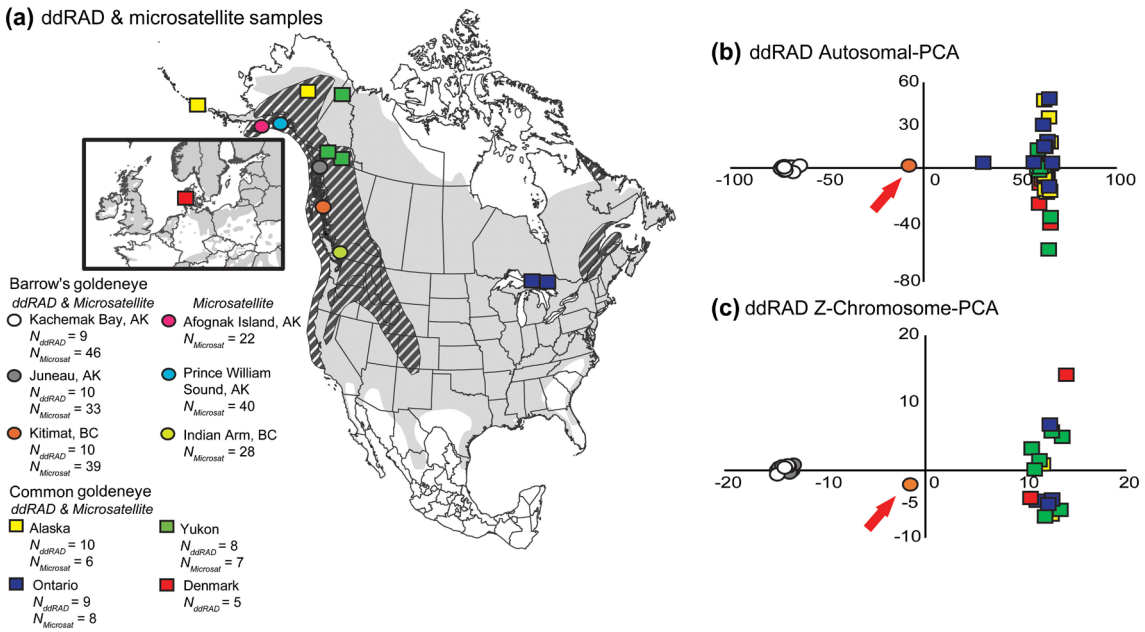

Figure 1. (a) Geographic distributions of common (grey) and Barrow's (hatched) goldeneye from North America and in Denmark (map inset; adapted from IUCN Red List and BirdLife International 2018a, b), including the location of Barrow's goldeneye and common goldeneye samples collected for double digest restriction-site associated DNA sequencing (ddRAD-seq) and microsatellite genotyping. (b) Autosomal and (c) Z-chromosome PCA results based on bi-allelic ddRAD single nucleotide polymorphisms (SNPs). Common goldeneye (squares) and Barrow's goldeneye (circle) create unique species' clusters in PCA results. Samples are color-coded by geographic region and the arrow denotes a putative F1 hybrid individual.

goldeneye ducklings (n = 1249) that were re-sighted (~3% of Barrow's goldeneye and ~2% of common goldeneye) were all female, and most (70% and 64% respectively) showed strong philopatry (i.e. returned to nest on their natal lake, Eadie et al. 1995, 2000). Additionally, mitochondrial DNA (mtDNA) was highly structured for both Barrow's goldeneye (F_{ST} = 0.313–0.921) and common goldeneye (among North American locales F_{ST} = 0.398–0.664), suggesting that female dispersal was significantly restricted (Pearce et al. 2014). The same study also showed no overlap in winter band recoveries among individuals marked in Alaska and British Columbia during the summer (Pearce et al. 2014). Additionally, satellite telemetry has shown high inter-annual winter site fidelity for Pacific Barrow's goldeneye (Willie et al. 2020). However, there was some overlap in recovery for common goldeneye marked in central Canada and eastern North America (Pearce et al. 2014), suggesting that common goldeneye exhibit dispersal to multiple wintering areas on both coasts. Given the higher levels of winter and breeding site fidelity exhibited by Barrow's goldeneye, we would expect to see greater levels of genetic structure relative to common goldeneye.

Both species of goldeneye commonly exhibit intra-specific egg dumping and inter-specific brood parasitism (Eadie and Fryxell 1992, Eadie et al. 1998), which has contributed to higher rates of incorrect mate pairing in other avifauna (e.g. ducks, Randler 2005; great tits *Parus major* and blue tits *P. caeruleus*, Slagsvold and Hansen 2001). However, strong territoriality and habitat partitioning on wintering, spring-staging and breeding grounds during mate-pairing may minimize opportunities for hybridization (pre-zygotic factors) and subsequent backcrossing (i.e. gene flow; Kraus et al. 2012). Although hybridization between goldeneyes has been documented in the wild (Ball 1934, Gray 1958, Martin and DiLabio 1994), identification of hybrids has relied largely on phenotypic characters. Due to phenotypic variation among sex and age classes in other waterfowl, the use of such characters alone has led to an overestimation of hybridization in some species (Williams et al. 2005; e.g. mottled duck × mallard hybridization, Bielefeld et al. 2016; Mexican duck × mallard hybridization, Hubbard 1977, Scott and Reynolds 1984). Therefore, while field observations suggest hybridization between goldeneye is rare, overall rates of hybridization and inter-specific gene flow are still largely unknown.

Here, we assess patterns of intra-specific genetic structure and diversity as well as gene flow between Barrow's goldeneye and common goldeneye at a regional scale by incorporating two different marker types (microsatellite markers and genome-wide SNPs derived from double digest restriction-site associated DNA sequencing (ddRAD-seq)). Specifically, we aim to test whether patterns of regional (Alaska, Yukon, Ontario) genetic differentiation within both species at autosomal and sex-linked (Z-chromosome) loci correspond to population structure identified by previous analyses of band recovery and maternally-inherited mtDNA data (Pearce et al. 2014). Given high wintering site fidelity (where pair formation likely occurs), high mtDNA differentiation (F_{ST} = 0.58; Pearce et al. 2014) and strong intra-specific nuclear genetic structure detected in other sea duck species (common eider, *Somateria mollissima*; Sonsthagen et al. 2011, but see Pearce et al. 2004, 2005, Wilson et al. 2016, Sonsthagen et al. 2019, 2020b), we expect to find some level of structure in the nuclear genome. Despite high breeding site fidelity, a lack of genetic structure in Barrow's goldeneye along the Pacific Coast or common goldeneye between sampling regions would suggest that wintering areas are likely used by individuals hatched from multiple nesting regions, or that an initial dispersal event of juveniles acts to homogenize genetic diversity. Finally, we aim to estimate contemporary and historical rates of gene flow between Barrow's goldeneye and common goldeneye. If strong breeding barriers have developed, then we expect to find few recent generation hybrids or backcrosses, and no or little (i.e. <1 migrant per generation) evidence of gene flow at the evolutionary scale.

Material and methods

Sampling, DNA extraction, ddRAD-seq library preparation, microsatellite DNA genotyping and mtDNA control region sequencing

Feathers, muscle or blood were collected as part of other research efforts from Barrow's goldeneye at winter or spring staging areas from 2009 to 2014 for both microsatellite (n = 208 total samples) and ddRAD (n = 32 total samples) sequencing. Specifically, samples were collected from Kachemak Bay (Alaska, USA; April; $N_{\text{ddRAD}} = 9$; $N_{\text{Microsatellite}} = 46$), Afognak Island (Alaska, USA; August; $N_{\text{Microsatellite}} = 22$), Prince William Sound (Alaska, USA; March; $N_{\text{Microsatellite}} = 40$), Juneau (Alaska, USA; April; $N_{\text{ddRAD}} = 10$; $N_{\text{Microsatellite}} = 33$), Kitimat (British Columbia, Canada; April; $N_{\text{ddRAD}} = 10$; $N_{\text{Microsatellite}} = 39$) and Indian Arm (near Vancouver in British Columbia, Canada; April; $N_{\text{Microsatellite}} = 28$; Fig. 1). Common goldeneye were collected opportunistically mainly from breeding summer birds, but also from some potential breeders in the spring, as well as from wintering sites, including: Alaska, USA (May–August $N_{\text{ddRAD}} = 8$, October–November $N_{\text{ddRAD}} = 2$; $N_{\text{Microsatellite}} = 6$), the Yukon Territory, Canada (April $N_{\text{ddRAD}} = 6$; June $N_{\text{ddRAD}} = 2$; $N_{\text{Microsatellite}} = 7$), Ontario, Canada (April $N_{\text{ddRAD}} = 2$; June–July $N_{\text{ddRAD}} = 7$; $N_{\text{Microsatellite}} = 8$) and Denmark (October $N_{\text{ddRAD}} = 5$), between 1998 and 2011 (Fig. 1). Samples of common goldeneye for this study from locales outside of Alaska were previously used in analyses by Pearce et al. (2014); Sonsthagen et al. (2020a) for additional sample information. Blood was stored on Whatman FTA cards or in blood preservation buffer (Longmire et al. 1988) at −80°C, muscle tissue was stored in a high-urea tissue preservation buffer (4.0 M urea, 0.2 M NaCl, 10 mM EDTA, 0.5% N-lauroyl-sarcosine and 100 mM tris–HCl) at −80°C, and feather samples were stored in coin envelopes at room temperature until further processing.

Genomic DNA was extracted from all sample types using a DNeasy blood & tissue kit following manufacturer protocols (Qiagen, Valencia, CA, USA). After ddRAD library

prep, all samples were pooled in equimolar amounts, and 150 bp, single-end sequencing was completed on an Illumina HiSeq 2500 at the Tufts University Core Genomics Facility. Specific protocols for ddRAD-seq library preparation followed DaCosta and Sorenson (2014, and also see Lavretsky et al. 2015, 2016, 2019) and are outlined in detail in Supplementary information. Raw Illumina reads were deposited in NCBI's sequence read archive (SRA; <http://www.ncbi.nlm.nih.gov/sra>; SRA data: SAMN15681561–SAMN15681621); Sonsthagen et al. (2020a) for ddRAD accession information by sample. Raw Illumina sequence reads were processed using the custom Python scripts designed by DaCosta and Sorenson (2014) (<http://github.com/BU-RAD-seq/ddRAD-seq-Pipeline>) and are outlined in detail in Supplementary information. Final output files were generated for downstream analyses (e.g. fasta, Nexus, ADMIXTURE) using custom python scripts (Lavretsky et al. 2016). Only loci with ≥ 5× coverage and < 10% missing data were retained for downstream analyses. Additionally, loci were categorized as either autosomal or Z-chromosome linked based on BLAST hits with only one unique match (Lavretsky et al. 2015).

Genomic DNA extractions and polymerase chain reaction amplification protocols for eight microsatellite loci (Aph02, Aph11, Maak et al. 2003; Aalμ1, Sfiμ4, Fields and Scribner 1997; Smo4, Smo7, Smo12, Paulus and Tiedemann 2003; and Sfiμ8, GenBank accession AF180498; F 5′-ATGATACAAATAAATAATAAAGCA-3′, R 5′-TGAAGACTGTGCTTATGAACTA-3′) followed protocols in Sonsthagen et al. (2004). Additionally, a more detailed outline of microsatellite genotyping protocols are included in Supplementary information and genotype data are available from Sonsthagen et al. (2020a).

A subset of Barrow's goldeneye were initially sequenced at the mtDNA control region to confirm the efficacy of primers and protocols previously developed by Pearce et al. (2014; Supplementary information). The resulting sequences suggested the presence of a nuclear pseudogene (Lopez et al. 1994, Sorenson and Fleischer 1996, Sorenson and Quinn 1998), and thus mtDNA sequencing was excluded from further analyses (Supplementary information).

Estimation of genetic diversity and population structure

For within- and between-goldeneye species analyses, pairwise locus-by-locus and composite estimates of relative divergence (Φ_{ST}), nucleotide diversity (π) and Tajima's D estimates were calculated each for autosomal and Z-linked ddRAD loci using the package PopGenome (Pfeifer et al. 2014) in the program R.

We calculated allelic richness, observed and expected heterozygosity, Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) for microsatellite loci in FSTAT 2.9.3 (Goudet 1995). Assessments of population genetic structure were conducted in ARLEQUIN 2.0 (Schneider and Excoffier 1999) by calculating overall and pairwise F_{ST}. Tests for HWE, LD and F_{ST} based on microsatellite data were corrected for multiple comparisons using Bonferroni correction ($\alpha = 0.05$).

For autosomal and Z-chromosome ddRAD loci, population genetic structure was estimated separately using a principal components analysis (PCA) as implemented by the package adegenet in the program R (Dray and Dufour 2007, Jombart 2008). For PCAs, we plotted only the first two principal components. For Z-linked markers, we only included male samples (n = 33) because PCA requires all individuals to be either haploid or diploid (in avian taxa, only males have two copies of the Z chromosome). Next, we used only autosomal ddRAD loci with the program ADMIXTURE (Alexander et al. 2009, Alexander and Lange 2011) to estimate the most likely number of populations (K) and individual assignment probabilities. Following steps outlined in Alexander et al. (2015), bi-allelic single nucleotide polymorphisms (SNPs) across ddRAD loci were formatted for analysis in plink (Purcell et al. 2007). We ran K of one to five populations in ADMIXTURE, with each K run 100 times and we employed a 10-fold cross-validation with a quasi-Newton algorithm across runs (Zhou et al. 2011). A block-relaxation algorithm was used for point-estimation, and analyses terminated once the change in the log likelihood of the point estimates increased by < 0.0001. The optimal K was based on the lowest average of CV-errors across all 100 replicates. We used the package PopHelper (Francis 2017) in R to convert all ADMIXTURE outputs into CLUMPP input files. Final assignment probabilities were based on the optimal clustering alignment across all 100 replicates for the optimum K using the GreedySearch algorithm for 1000 iterations as implemented in the program CLUMPP v.1.1 (Jakobsson and Rosenberg 2007).

For microsatellite loci, we used the Bayesian clustering program STRUCTURE 2.3.2 (Pritchard et al. 2000, Hubisz et al. 2009) to assign individuals to clusters based on allelic frequencies and infer the occurrence of genetic structure without a priori knowledge of putative populations. Data were analyzed using an admixture model assuming correlated frequencies and sample location information as a prior with a 50 000 burn-in period, followed by 500 000 Markov Chain Monte Carlo iterations, and number of possible populations ranging from 1 to 6; this analysis was repeated 10 times to ensure consistency across runs. We performed this analysis on each species separately and species combined to detect any signature of hybridization.

Inference of intra-specific gene flow

With the ddRAD loci, we used fineRADstructure (Malinsky et al. 2018) to infer population structure based on shared ancestry and gene flow. Briefly, fineRADstructure uses recent coalescent events to infer relatedness among samples and is informative in cases of recent and contemporary gene flow. Based on our results from ADMIXTURE, we performed a coancestry analysis on a combined common goldeneye and Barrow's goldeneye dataset, as well as with each species

separately. We concatenated all ddRAD loci, and bi-allelic SNPs were formatted for analysis in plink and using custom python scripts (Purcell et al. 2007, Lavretsky et al. 2016). Samples were assigned to populations using 1 000 000 iterations sampled every 1000 steps with a burn-in of 100 000. We then used 100 000 iterations of the tree-building algorithm to assess genetic relationships among clusters. Finally, results were visualized using the R scripts fineradstructureplot.r and finestructurelibrary.r (available at <http://cichlid.gurdon.cam.ac.uk/fineRADstructure.html>).

Estimating inter-specific gene flow, effective population sizes and time since divergence

We tested for and estimated rates and directionality of gene flow using the program ∂a∂i (Gutenkunst et al. 2009, 2010), which uses a diffusion-based approach to test empirical data against specified evolutionary models (e.g. isolation-with-migration; Supplementary information). Briefly, ∂a∂i determines the best fit evolutionary model using a site-frequency spectrum derived from bi-allelic SNPs. Using a common goldeneye/Barrow's goldeneye joint site-frequency spectrum, we tested three evolutionary models including isolation-with-migration, split-migration (i.e. recurring secondary contact) and neutral-no-divergence (Supplementary information). We used the best fit model as determined by ∂a∂i to calculate the optimum parameters as well as uncertainty metrics (i.e. standard deviation; Gutenkunst et al. 2009, Coffman et al. 2016). Different demographic parameters were simultaneously estimated by ∂a∂i, including θ (i.e. molecular diversity; $\theta = 4N_{Anc} \times \mu$; N_{Anc} = ancestral effective population size), effective population sizes ($N_i = n_i \times N_{Anc}$), migration levels ($M = 2N_{Anc} \times m$) and divergence times ($t = T \times 2N_{Anc}$; Gutenkunst et al. 2009). Finally, we used a generation time of 4.03 years and mutation rate of 2.19×10^{-3} substitutions/site/generation (s/s/g) to convert ∂a∂i parameters into biologically informative values (Supplementary information for more detailed methods).

Results

ddRAD data

After quality filtering the ddRAD dataset, we recovered 3678 loci, with 3502 (n = 451 951 bp) and 176 (n = 22 738 bp) loci assigned to autosomes and the Z-chromosome, respectively (Supplementary information). An average median sequencing depth of 94 reads per locus per individual (range = 27–320 reads/locus/individual) was obtained across samples.

Estimates of genetic diversity

Overall π at ddRAD autosomal markers for common goldeneye (π = 0.0039) was ~3 times higher than for Barrow's goldeneye (π = 0.0014; Table 1). Furthermore, π showed minimal variation across sampling sites in either common goldeneye (π = 0.0038–0.0039) or Barrow's goldeneye (π = 0.0011–0.0017). Similar patterns were observed at Z-chromosome markers, where common goldeneye (π = 0.0018) had ~2 times higher π than Barrow's goldeneye (π = 0.00081; Table 1). Moreover, Tajima's *D* did not significantly deviate from zero across common goldeneye sample sites in ddRAD autosomal or Z-linked markers (Table 1). In contrast, Tajima's *D* for Barrow's goldeneye was negative for both autosomal (Tajima's $D = -1.6$) and Z-linked markers (Tajima's $D = -1.9$; Table 1), suggestive of population growth. Indices of genetic diversity based on microsatellites were similar across sampled sites for Barrow's goldeneye and common goldeneye (Table 1).

Intra-specific comparisons of genetic structure and gene flow

With ddRAD loci, there was no evidence of genetic structure within either Barrow's goldeneye (autosomal $\Phi_{ST} = -0.0017$ to -0.00094, Z-chromosome $\Phi_{ST} = -0.025$ to 0.011) or common goldeneye (autosomal $\Phi_{ST} = 0.0032$–0.016, Z-chromosome $\Phi_{ST} = 0.012$–0.029), even between North

Table 1. Sample sizes (*N*) and indices of genetic diversity including the mean number of alleles (A), allelic richness (AR) and observed and expected heterozygosity (H_o/H_e in %) based on 7–8 microsatellite loci, as well as nucleotide diversity (π) and Tajima's *D* using double digest restriction-site associated DNA sequencing (ddRAD-seq) autosomal and Z-chromosome loci for Barrow's goldeneye and common goldeneye sampled locations.

	Microsatellite					ddRAD – autosomal			ddRAD – Z	
	N	A	AR	H_o	H_e	*N*	π	*D*	π	*D*
Barrow's	208	–	–	–	–	29	0.0014	−1.6	0.00081	−1.9
Kachemack Bay	46	4.7	4.1	45.2	46.0	9	0.0012	−0.5	0.00070	−1.0
Juneau	33	4.6	4.1	50.2	51.3	10	0.0011	0.0	0.00063	−0.3
Kitimat	39	5.2	4.3	45.1	50.0	10	0.0017	−1.5	0.0011	−1.7
Afognak	22	4.1	4.1	47.9	48.1	–	–	–	–	–
Prince William Sound	40	5.0	4.3	47.9	49.5	–	–	–	–	–
Indian Arm	28	4.1	3.9	40.5	48.9	–	–	–	–	–
Common	21	–	–	–	–	32	0.0039	−0.3	0.0018	−0.8
Alaska	6	3.7	3.7	42.9	61.5	10	0.0038	−0.1	0.0019	−0.2
Yukon	7	3.7	3.6	51.0	63.4	8	0.0038	−0.1	0.0017	−0.5
Ontario	8	3.6	3.4	48.2	58.2	9	0.0038	−0.3	0.0018	−0.5
Denmark	–	–	–	–	–	5	0.0039	−0.1	0.0018	−0.1

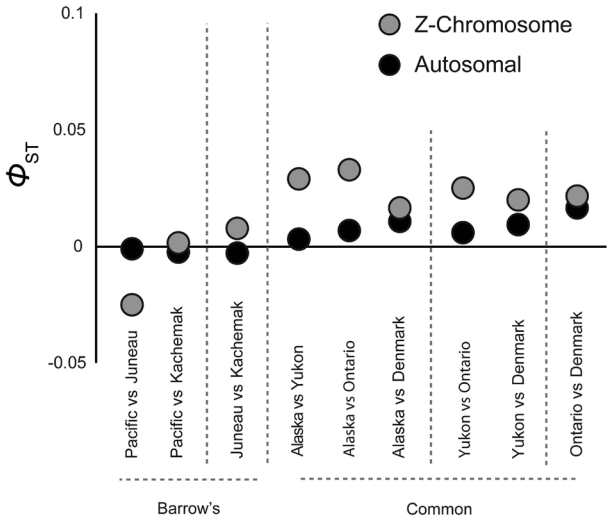

Figure 2. Composite pairwise Φ_{ST} estimates for 3502 autosomal and 176 Z-chromosome ddRAD loci between common goldeneye and Barrow's goldeneye sampled locations.

American and European common goldeneye (autosomal $\Phi_{ST}=0.0095$–0.016, Z-chromosome $\Phi_{ST}=0.016$–0.019; Fig. 2). Population structure was visualized using a dataset that included 13 154 bi-allelic ddRAD-seq autosomal SNPs. Concordant with the low pair-wise Φ_{ST} estimates (Fig. 2), ADMIXTURE recovered no sub-structuring within Barrow's goldeneye or within common goldeneye ($K=1$ for both species; Supplementary information). Whereas the combined common goldeneye and Barrow's goldeneye fineRADstructure uncovered some sub-structuring within species, samples within these groups were not clustered by geographic region, suggesting high rates of gene flow among sampled locales (Fig. 3). The within-Barrow's goldeneye analysis returned similar results; sub-structuring was not associated with geographic regions, and analysis with only common goldeneye did not uncover clustering of any individuals (Supplementary information).

Microsatellite loci did not deviate from HWE and were not in LD. Like the ddRAD loci, spatial patterns in allelic frequencies were not uncovered among sampled sites at microsatellite loci for Barrow's goldeneye (8 microsatellite loci: overall $F_{ST}=0.0014$, $p=0.30$; pairwise $F_{ST}=-0.0055$ to 0.010; $p > 0.24$) or for common goldeneye (7 microsatellite loci: overall $F_{ST}=0.015$, $p=0.20$; pairwise $F_{ST}=0.0.0002$–0.0264; $p > 0.06$). Individual species STRUCTURE and ADMIXTURE analyses both failed to uncover geographically based differentiation (below). Despite finding that $K=2$ (LnP|K$=-3172$, ΔK$=54.1$, r < 1.0; $K=1$, LnP|K$=-3193$) is the most likely number of clusters in Barrow's goldeneye based on microsatellite loci in STRUCTURE, samples within these clusters did not group by geographic locality. Genetic structure was not uncovered within common goldeneyes ($K=1$, LnP|K$=-352$, r < 1.0; $K=2$, LnP|K$=-354$). The combined Barrow's goldeneye and common goldeneye analysis also recovered $K=2$ as the most likely number of clusters given the data (LnP|K$=-3529$, ΔK$=737.3$, r < 1.0; $K=1$, LnP|K$=-4093$; Fig. 3).

Inter-specific comparisons of population structure and gene flow

With ddRAD loci, strong between species population structure was recovered between common goldeneye and Barrow's goldeneye for autosomal (composite $\Phi_{ST}=0.53$) and Z-linked loci (composite $\Phi_{ST}=0.67$). Moreover, comparing locus-by-locus Φ_{ST} estimates revealed some nearly fixed differences between the two species (i.e. $\Phi_{ST} > 0.95$; $N_{\text{Autosomal}}=93$ and $N_{\text{Z-Chromosome}}=16$; Supplementary information). The strong structure observed between Barrow's goldeneye and common goldeneye was also detected by ADMIXTURE when analyzing 13 154 bi-allelic autosomal SNPs, where $K=2$ was the optimum number of clusters (Supplementary information). Higher values of K failed to uncover additional structure (Fig. 3). Under a two-population model, a single F1 hybrid individual was identified (assignment probability of 0.519 to the Barrow's goldeneye cluster), while the remaining samples were assigned to their respective species clusters with >99% probability (Fig. 3). The putative F1 hybrid identified using ddRAD autosomal data also showed high assignment to the Barrow's goldeneye cluster (0.60 assignment) identified in STRUCTURE when using a larger number of microsatellite samples (Fig. 3). Next, ddRAD autosomal and Z-linked PCA results were both concordant with ADMIXTURE; the F1 hybrid was found to be intermediate between the respective common goldeneye and Barrow's goldeneye species' clusters (Fig. 1) and shared nearly equal coancestry with both species in fineRADstructure (Fig. 3). Finally, greater variation among common goldeneye samples in the PCA is consistent with their higher nucleotide diversity (Table 1) and larger effective population size (below).

Estimating gene flow, divergence time and effective population size

The same set of bi-allelic ddRAD autosomal SNPs as used for ADMIXTURE analyses were used in our ∂a∂i analyses. Concordant with evidence of recent hybridization found in both ddRAD and microsatellite datasets, likelihood estimates supported a two population split-with-migration evolutionary model as the optimum (estimated likelihoods: neutral-no-divergence$=-70$ 032; isolation-with-migration$=-3043$; isolation-without-migration$=-5652$; split-with-no-migration$=-2412$; split-with-migration$=-1101$). Using a mutation rate of 2.19×10^{-3} s/s/g to convert ∂a∂i results into biologically meaningful variables, we calculated effective population size to be ~3.5 times higher in common goldeneye (average $N_e=226$ 419; 95% CI$=217$ 256–235 582) relative to Barrow's goldeneye (average $N_e=63$ 949; 95% CI$=62$ 820–65 078), and an average time since divergence of ~1 660 305 years before present (95% CI$=1$ 630 743–1 689 868 years before present). Though our optimum evolutionary model recovered evidence for bi-directional

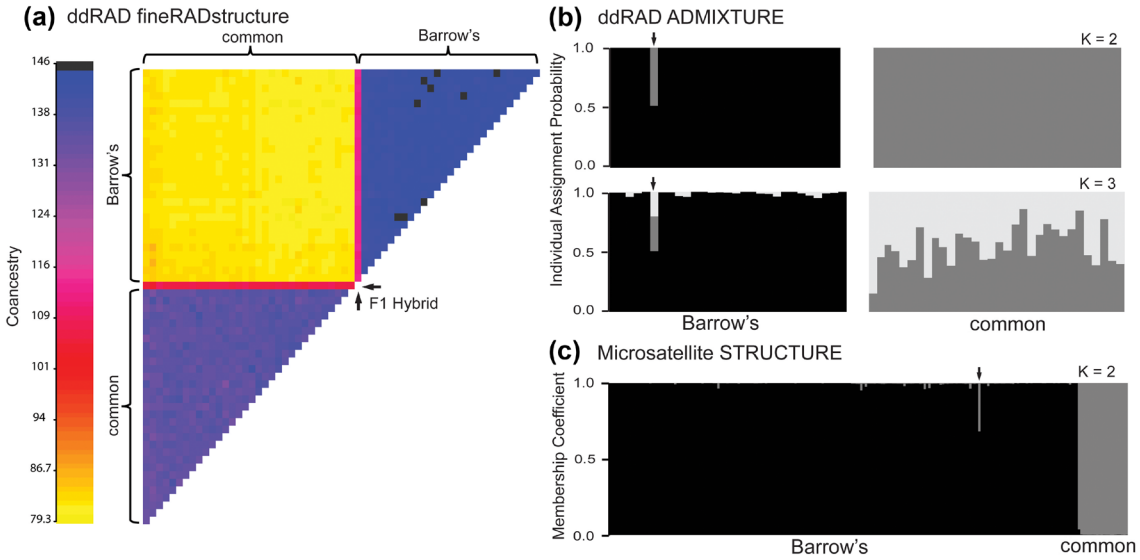

Figure 3. (a) fineRADstructure coancestry plot for Barrow's goldeneye and common goldeneye based on 13 154 autosomal double digest restriction-site associated DNA (ddRAD) bi-allelic single nucleotide polymorphisms (SNPs). (b) Maximum-likelihood estimation of individual assignment probabilities from ADMIXTURE (Alexander et al. 2009) for $K=2$–3 populations based on autosomal ddRAD markers. (c) STRUCTURE assignment membership coefficients for $K=2$ clusters based on 7–8 microsatellite markers. Arrow denotes a putative F1 hybrid individual.

gene flow ($m_{BAGO–COGO}=0.079$ migrants/generation (95% CI = 0.071–0.088 migrants/generation); $m_{COGO–BAGO}=0.022$ migrants/generation (95% CI = 0.021–0.024 migrants/generation)), all estimates were <<1 migrant per generation.

Discussion

Nuclear variation recovers no intra-specific genetic structure in goldeneye species

Here, we provide the first genomic assessment of common goldeneye and Barrow's goldeneye populations that examines regional genetic differences encompassing wintering and spring breeding sites and measures gene flow between the two species. We found no discernible structure within either species across three different marker types (i.e. autosomal microsatellites, autosomal ddRAD-seq and Z-chromosome ddRAD-seq). This contrasts with previous research based on mtDNA and band-recovery data that shows strong regional-level structure within both species across North America (Pearce et al. 2014). Such mito-nuclear discord is often attributed to aspects of species breeding biology; specifically, female philopatry maintains mtDNA structure, while male-biased dispersal homogenizes the nuclear genome (e.g. common merganser *Mergus merganser*, Pearce and Petersen 2009, Pearce et al. 2009; common eider *Somateria mollissima*, Sonsthagen et al. 2011; spectacled eider *S. fisheri*, Scribner et al. 2001; Steller's eider *Polysticta stelleri*, Pearce et al. 2005).

However, if males are the primary dispersers in goldeneye, and dispersal occurs during migration (when pair bonding occurs), then the inclusion of males from both breeding and wintering grounds by Pearce et al. (2014) makes it difficult to identify the processes promoting this apparent structure in mtDNA. We posit that the mtDNA structure could have been a temporary signal as male mtDNA haplotypes are not passed onto the next generation (albeit this happens rarely), and that a female only assessment that distinguishes between patterns of population structure on breeding and wintering grounds is necessary to confirm which sex (if either) is the primary dispersing agent over a broad time scale. Finally, given that telemetry data suggest females and to some extent adult males display high regional fidelity (Willie et al. 2020), we contend that males, more likely immature males, are the primary dispersing agent maintaining nuclear similarity across each species' respective ranges.

The lack of genetic structure among geographic regions within Barrow's goldeneye (Alaska versus British Columbia) differs from patterns detected using band-recovery data (Pearce et al. 2014), which distinguished distinct wintering regions along the Pacific Coast for individuals banded during the summer. Male-biased dispersal does not fully explain the observed patterns of discord as Barrow's goldeneye exhibit high rates of breeding site fidelity in both sexes, with the average yearly return rate for adult males (67% ± 11%) nearly identical to that of females (63% ± 4%; Savard and Eadie 1989). The high fidelity of adult males to nesting areas may result from long-term pair bonds exhibited by Barrow's goldeneye, as both sexes have high wintering site fidelity (Willie et al. 2020). Additionally, male Barrow's goldeneye vigorously defend territories and are therefore more likely to reunite on the wintering grounds where mate selection occurs (Savard 1985). While strong nesting and wintering-site fidelity exhibited by adult male Barrow's goldeneye likely drives partitioning observed in

band-recovery data, we contend that homogeneity across the nuclear genome most likely results from high levels of juvenile male dispersal despite high mtDNA structure (above). Telemetry data for Barrow's goldeneye are consistent with this conclusion and patterns seen in other sea ducks (e.g. king eider, Oppel and Powell 2010, Bentzen and Powell 2015; common merganser, Pearce and Petersen 2009), and specifically indicate that subadult males return to natal nesting grounds significantly less often than subadult females and are much more likely to disperse across wintering grounds than pre-breeding females (Boyd et al. 2009).

While overall rates of female philopatry remain low in common goldeneye, individuals returning to natal breeding grounds often dispersed < 1 km from previous nesting sites (Dow and Fredga 1983, Pöysä et al. 1997). However, rates of philopatry and dispersal varied between study sites and may reflect localized patterns (Lawson et al. 2017). Although there are no data on male dispersal in common goldeneye, the general lack of genetic structure within and between Eurasia and North America suggests that common goldeneye likely comprise a single panmictic population across continents, despite some morphological differences (Palmer 1976, Bellrose 1980). Once again this finding is in contrast to all other Holarctic sea duck species for which range-wide genomic structure has been assessed (e.g. common eider nuclear introns $\Phi_{ST} = 0.000–0.208$, Sonsthagen et al. 2011; common merganser nuclear introns $\Phi_{ST} = 0.254–0.274$, Peters et al. 2012; black scoter *Melanitta americana* ddRAD $\Phi_{ST} = 0.196–0.200$, white-winged scoter *M. deglandi* ddRAD $\Phi_{ST} = 0.155–0.160$, Sonsthagen et al. 2019). The finding that common goldeneye appears unstructured across the Atlantic Ocean suggests that dispersal between Eurasia and North America is potentially more common than previously thought. However, sea ducks show differences in movement patterns across demographic classes (e.g. king eider; Oppel and Powell 2010, Bentzen and Powell 2015), and data on male versus female dispersal distances are limited; therefore, future work examining connectivity among regions within common goldeneye would benefit from telemetry data collected across all age and sex classes. Finally, we note that while juvenile male dispersal is most likely responsible for homogenizing the nuclear genome across geographic regions, populations may be demographically structured at smaller spatial scales not indicated by genetic analyses.

Nuclear variation recovers strong structure and limited gene flow between the goldeneye species

Although being sister taxa, Barrow's goldeneye and common goldeneye are strongly differentiated across multiple marker-types (Fig. 2), including many nuclear loci that are effectively fixed (Supplementary information), and are also clearly structured (Fig. 1, 3). Despite such strong population structure, we expected some level of hybridization and gene flow because both species exhibit inter-specific nest parasitism, which is known to promote incorrect imprinting and future mate pairing (Randler 2005, Balakrishnan et al. 2008); nevertheless, we find limited evidence for both. First, only a single common × Barrow's goldeneye hybrid was recovered in both ddRAD-seq (n = 1/63, 1.6%) and microsatellite (n = 1/229, 0.4%) datasets. Although this suggests that limited hybridization does occur, the admixed individual was identified as a first generation (F1) hybrid (Fig. 1, 3), and a further lack of identifiable backcrossed individuals suggests that interspecific gene flow is restricted. This pattern is supported by estimates of gene flow from demographic ∂a∂i analyses. Specifically, while an optimum evolutionary model of split-with-migration was recovered for goldeneye, the estimated rate of bi-directional gene flow was << 1 migrant per generation. In contrast, scaup *Aythya* spp., another pair of sister sea duck taxa, showed evidence of only a few late-generation backcrosses and no contemporary hybrids (Lavretsky et al. 2016). We contend that the limited level of gene flow between goldeneye, which has likely occurred during bouts of secondary contact, has been ineffective at eroding genomic barriers between the two species (Supplementary information). The lack of gene flow also suggests that strong pre-zygotic barriers have evolved between these two species, potentially including assortative mating, differences in habitat preferences and territorial behaviors exhibited during mate pairing (Johnsgard 1978, Savard 1984, 1985, 1988, del Hoyo et al. 1992). Specifically, common goldeneye prefer low elevation freshwater ponds, while Barrow's goldeneye prefer high elevation (> 610 m) alkaline ponds (Savard et al. 1994, Robert et al. 2008). This habitat partitioning in addition to strong territoriality exhibited by males during mate pairing may be sufficient to prevent interspecific pairings (Hagen and Taylor 2001, Ostberg et al. 2004), even in cases of nest parasitism.

Finally, we estimated an overall divergence time between common goldeneye and Barrow's goldeneye of ~1.6 million years before present, which falls within previous estimates based on mtDNA (~2.0 MYA; range = 1.3–2.8 MYA; Fulton et al. 2012, Jetz et al. 2012, Kumar et al. 2017). Moreover, recovery of a Z-chromosome to autosomal Φ_{ST} ratio of 1.27 (Fig. 2) is consistent with the genomes of these two species being under similar evolutionary constrains. This ratio indicates an evolutionary scenario in which the genomes of Barrow's goldeneye and common goldeneye have largely diverged neutrally and in allopatry (where we expect a Z-chromosome to autosomal Φ_{ST} ratio of ≤ 1.33; Caballero 1995, Whitlock and McCauley 1999, Dean et al. 2015), as is also the case between other sister taxa of scaup (Lavretsky et al. 2016) and scoters (Sonsthagen et al. 2019). Thus, here we provide evidence that the divergence of the two goldeneye species is likely the result of a relatively deep evolutionary split, and that these two species have likely been evolving under neutral conditions and largely independent from one another throughout their evolutionary histories.

Comparing goldeneye census versus effective population sizes

Common goldeneye show significant deviations between estimated census size (2 700 000–4 700 000; Butcher and Niven

2007, Wetlands International 2015) and estimated effective population size (N_e = 226 419; 95% CI = 217 256–235 582). While the lowered effective population/census (N_e/N) size ratio of ~0.06 found for common goldeneye suggests recent fluctuations in population size (Vucetich et al. 1997), long term survey trends (Wetlands International 2015) and the near zero estimates of Tajima's D for autosomal (Tajima's $D = -0.3$) and Z-chromosome markers (Tajima's $D = -0.7$) suggest that effective populations have been stable. Thus, we conclude that the deviation in the N_e/N ratio for common goldeneye is likely due to variance in demographic factors (Vucetich et al. 1997). Specifically, sea ducks are known to have skewed sex ratios (Johnsgard and Buss 1956, Duncan and Marquiss 1993), overlapping generations and spatially and temporally variable fecundity (Milonoff et al. 1998, 2004), all of which contribute to a lowered N_e/N ratio (Vucetich et al. 1997). In contrast, ∂a∂i analyses for Barrow's goldeneye estimated an effective population size of 63 949 individuals (95% CI = 62 820–65 078), which is ~0.28 of current census estimates of 205 000–258 000 (Butcher and Niven 2007, Wetlands International 2018). While a N_e/N ratio that falls between 0.25 and 0.75 is consistent with the long-term population stability characterizing a majority of the Barrow's goldeneye's range (Eadie et al. 2000), this N_e/N ratio is not consistent with the significantly negative Tajima's D calculated for autosomal (Tajima's $D = -1.6$) and Z-chromosome linked (Tajima's $D = -1.9$) markers, which suggest recent population expansion. We hypothesize that deviation between effective and census population sizes found here is also due to demographic factors. More localized information about demography (i.e. sex ratios, generation times and fecundity) is needed to better understand the effects of long-term population stability in common goldeneye and Barrow's goldeneye on their respective effective population sizes.

Conservation implications

Here, our findings highlight the importance of multi-marker comparisons and integrating these with more standard field approaches (Sork et al. 1999, Robertson et al. 2018). In doing so, researchers can generate a more comprehensive view of movement and dispersal patterns of individuals within and between species that would otherwise be overlooked. Specifically, genetic markers in conjunction with direct measures of movement, migratory connectivity and dispersal will be particularly useful for understanding the degree of contemporary demographic independence between geographic regions. In common goldeneye and Barrow's goldeneye, discord in estimates of population structure between data types (i.e. telemetry, banding and genetic markers) suggests that subadult males are likely the primary dispersing agents and are playing a disproportionate role in maintaining genetic connectivity between geographic regions. Additionally, as the availability and distribution of goldeneye habitat continues to change, understanding rates and patterns of gene flow will be critical in maintaining connectivity among nesting areas.

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Data availability statement

Input data files for downstream analyses (FASTA, ADMIXTURE, PCA, ∂a∂i and fineRADstructure): Dryad accession <https://doi.org/10.5061/dryad.kd51c5b3w> (Brown et al. 2020).

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